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STABILITY INDICATING METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF DEXTROMETHORPHAN HBr, PHENYLEPHRINE HCl AND CHLORPHENIRAMINE MALEATE IN THEIR COMBINED SYRUP DOSAGE FORM BY REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

The present work describes a validated reverse phase high performance liquid chromatographic method for simultaneous estimation of Dextromethorphan HBr, Phenylephrine HCl and Chlorpheniramine Maleate in syrup dosage form. The quantification was carried out using Phenomenex Luna 5µm C8(2)100Å;250×4.6mm (Part No.00G-4249-E0) column and mobile phase comprised of Buffer pH 3.2 and Acetonitrile with gradient elution mode. The flow rate was 1.0 ml/min and the eluent was monitored at 220 nm. The selected chromatographic conditions were found to effectively separate Dextromethorphan HBr, Phenylephrine HCl and Chlorpheniramine Maleate were about 11.1 min, 3.7 min and 10.3 min respectively. Linearity were found to be in the range of 80-120 µg/ml, 40-60 µg/ml and 16-24 µg/ml for Dextromethorphan HBr, Phenylephrine HCl and Chlorpheniramine Maleate respectively. The percentage recoveries of all the drugs were found to be 100.5-101.8%, 99.2-101.8% and 100.1-101.8% for Dextromethorphan HBr, Phenylephrine HCl and Chlorpheniramine Maleate. The proposed method was found to be fast, specific, accurate, precise, and reproducible and can be used for simultaneous estimation of these drugs in syrup formulation.

Keywords: Dextromethorphan HBr, Phenylephrine HCl, Chlorpheniramine Maleate, Reversed-phase HPLC.

INTRODUCTION

Dextromethorphan is a medication most often used as a cough suppressant in over-the-counter cold and cough medicines. It is sold in syrup, tablet, spray, and lozenge forms. It is in the morphinan class of medications with sedative, dissociative, and stimulant properties (at lower doses). Dextromethorphan does not have a significant affinity for the mu-opioid receptor activity typical of morphinan compounds and exerts its therapeutic effects through several others. Phenylephrine is a medication primarily used as a decongestant, to dilate the pupil, to increase blood pressure, and to relieve hemorrhoids. While marketed as a decongestant, taken by mouth at

recommended doses it is of unclear benefit for hay fever. It can be taken by mouth, given by injection into a vein or muscle, or applied to the skin.Common side effects when taken by mouth or injected include nausea, headache, and anxiety. Use on hemorrhoids is generally well tolerated. Severe side effects may include a slow heart rate, intestinal ischemia, chest pain, kidney failure, and tissue death at the site of injection. It is unclear if use during pregnancy or breastfeeding is safe. Phenylephrine is a selective α_1-adrenergic receptor activator which results in the constriction of both arteries and veins.

Chlorpheniramine (CP, CPM), also known as Chlorphenamine, is an antihistamine used to treat the symptoms of allergic conditions such as allergic rhinitis (hay fever). It is taken by mouth. The medication takes effect within 6 hours and lasts for about a day. Common side effects include sleepiness, restlessness, and weakness. Other side effects may include dry mouth and wheeziness. It is a first-generation antihistamine and works by blocking the H1 receptor. Chlorphenamine is often combined with phenylpropanolamine to form an allergy medication with both antihistamine and decongestant properties, though phenylpropanolamine is no longer available in the US after studies showed it increased the risk of stroke in young women. Chlorphenamine remains available with no such risk. The antihistamine is helpful in cases where allergy or common cold is the reason for the cough; it is also a potentiator of opioids, allowing enhanced suppression of cough, analgesia, and other effects from a given quantity of the drug by itself. In various places in the world, cough and cold preparations containing codeine and chlorphenamine are available. The main objectives of this work are to develop a simple and precise chromatographic stability indicating method of multi-components in syrup dosage form and to validate developed method by using different validation parameters.

MATERIALS AND METHODS

Standard, Sample, Reagents and Chemicals

Dextromethorphan HBr, Phenylephrine HCl and Chlorpheniramine Maleate working standard, In-house developed syrup formulation (Dextromethorphan Hydrobromide 10mg/5ml, Phenylephrine Hydrochloride 5mg/5ml, Chlorpheniramine Maleate 2mg/5ml), Acetonitrile of HPLC grade, Triethyl amine of HPLC grade, Octane-1-Sulfonic Acid Sodium Salt , Ortho Phosphoric Acid (88%) and water HPLC grade.

Instruments and Chromatographic Conditions

Shimadzu LC 2010 HPLC system with PDA detector was used for method development, degradation studies and validation. Data acquisition was performed on LC solution software. The separation were achieved on Phenomenex Luna 5µm C8(2)100Å ;250×4.6 mm column. The column was maintained at room temperature and the eluent was monitored at 222 nm using UV detector. Buffer pH 3.2 (mobile phase A) and Acetonitrile (mobile phase B) with gradient mode at a flow rate of 1.0 ml/min was used as a mobile phase. The injection volume was 20µl.

Preparation of Buffer

Dissolve 1.08 gm of Octane-1-Sulfonic Acid Sodium Salt and 1.0 mL of Triethylamine in to 1000 mL of water. Adjust pH 3.2 using 10% v/v Orthophosphoric acid solution in water. Filter the buffer with 0.22 µ filter paper.

Preparation of Mobile Phase

Mobile Phase A – Buffer pH 3.2
Mobile Phase B – Acetonitrile
Gradient:

Time (min)	Mobile Phase A (%)	Mobile Phase B (%)
0	70	30
2	70	30
12	40	60
13	20	80
16	20	80
17	70	30
22	70	30

Preparation of Diluent

Mix Buffer pH 3.2 and Acetonitrile in the ratio of 50:50 (v/v).

Standard Preparation

Standard Stock for Dextromethorphan HBr [A]

Weigh and transfer 100 mg of Dextromethorphan HBr working standard or reference standard in 100 mL of volumetric flask. Add 70 mL of Diluent. Sonicate it to dissolve. Make up the volume up to mark with diluent and mix well.

Standard Stock for Phenylephrine HCl [B]

Weigh and transfer 50 mg of Phenylephrine HCl working standard or reference standard in 100 mL of volumetric flask. Add 70 mL of Diluent. Sonicate it to dissolve. Make up the volume up to mark with diluent and mix well.

Standard Stock for Chlorpheniramine Maleate [C]

Weigh and transfer 40 mg of Chlorpheniramine Maleate working standard or reference standard in 200 mL of volumetric flask. Add 150 mL of Diluent. Sonicate it to dissolve. Make up the volume up to mark with diluent and mix well.

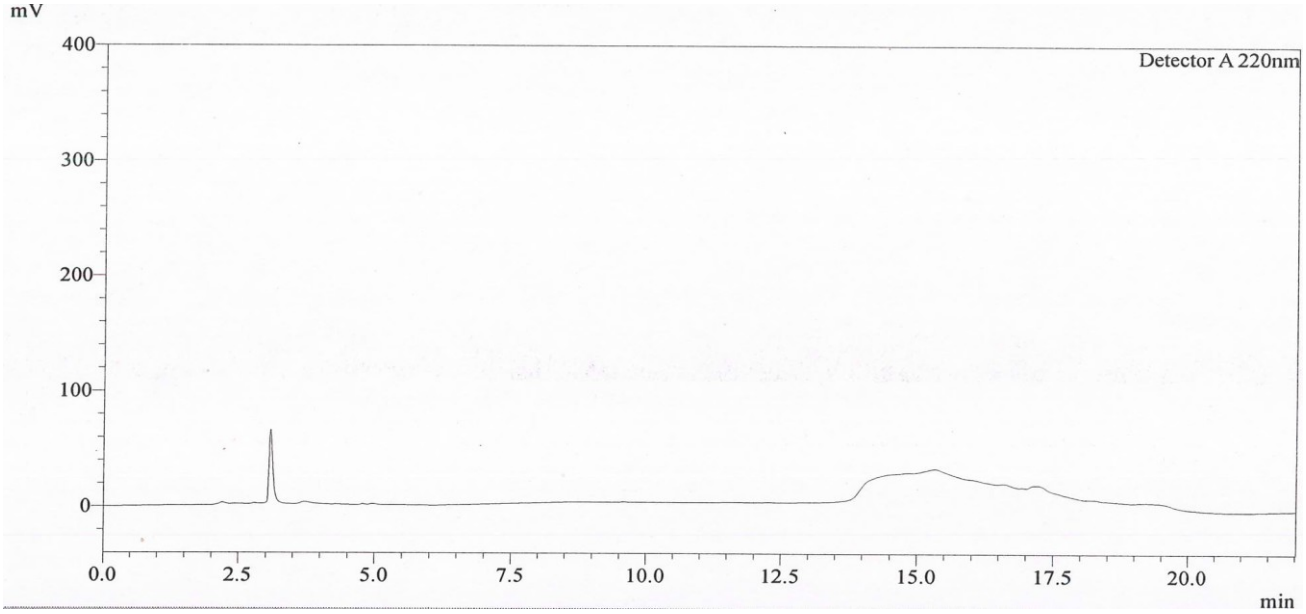
Preparation of Standard solution

Dilute 5 mL from each standard stock solution A, B and C into a 50 mL volumetric flask. Make up the volume with diluent and mix well. Filter the solution through whatman filter paper 0.45µ.

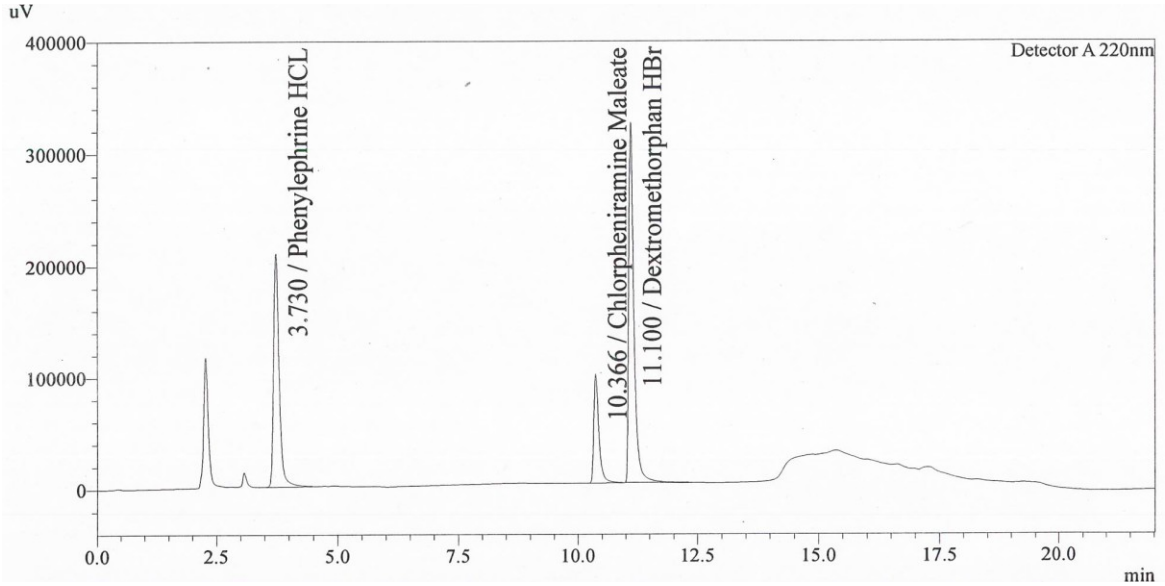
Test Preparation

Weigh and transfer about 6 gm of sample into 100 mL of volumetric flask. Add 80 mL of Diluent and sonicate for 15 minutes with intermittent shaking. Make up the volume up to the mark with the Diluent. Mix well and filter through whatman filter paper 0.45µ.

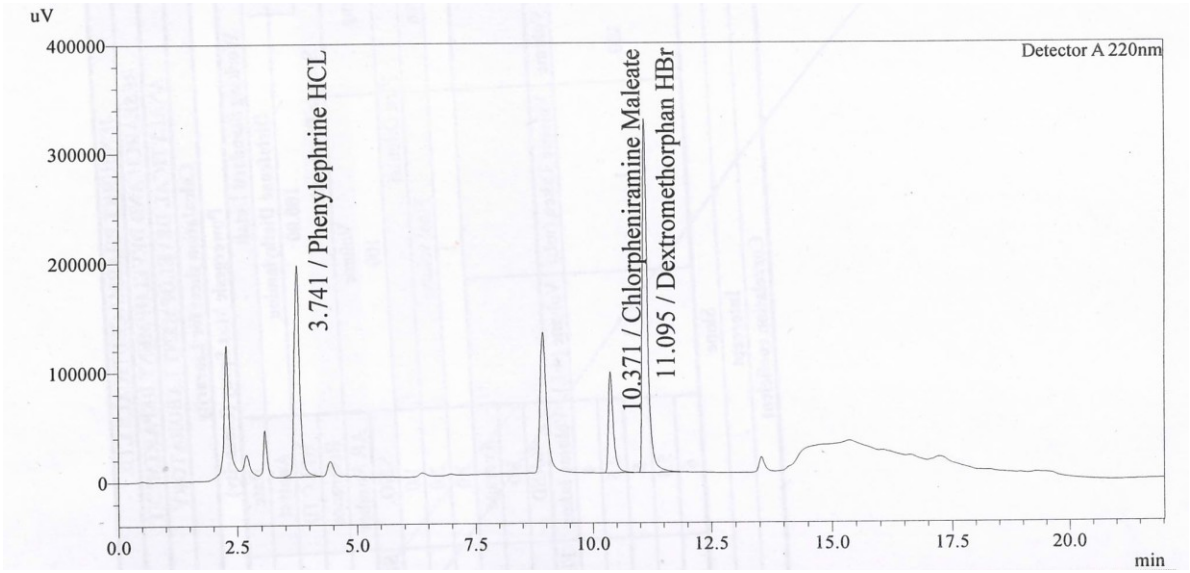
Typical chromatogram of Blank Solution



Typical chromatogram of Standard Solution



Typical chromatogram of Sample Solution



VALIDATION OF RP-HPLC METHOD

System Precision

Six replicate injections of standard solution were injected in to the chromatography system. %RSD for area response of individual peak of each drug component in six injections are caluculaed.

Specificity

Blank, standard and placebo solution were prepard and analyzed as per methodology.Sample were degraded at different stress condition and peak purity of active components peak are evaluated.

Method precision

Six homogeneous samples were prepared and analyzed as per methodology. % RSD for assay of Dextromethorphan HBr, Phenylephrine HCl & Chlorpheniramine Maleate in six replicate preparations were calculated.

Accuracy

Recovery experiment was performed for assay method of Dextromethorphan HBr, Phenylephrine HCl and Chlorpheniramine Maleate at three level considering 80% 100% and 120% of target concentration. Calculated % individual recovery and Mean Recovery.

Linearity

Linearity Experiment was performed for assay method of Dextromethorphan HBr, Phenylephrine HCl and Chlorpheniramine Maleate by preparing five standard solutions in concentration range of 80% to 120% of target concentration. A Graph was plotted between concentration and area responses.

Ruggedness (Intermediate Precision)

Six Homogeneous samples were prepared by different analyst, different day , different column and different instrument and injected into chromatoxgraphy system. % RSD for assay of each drug component in six replicate preparations were calculated.

Robustness

Following parameters were changed one by one and their effect was observed on system suitability for standard preparation.

1. Flow rate of mobile phase was changed (± 0.2 ml/min) 0.8 ml/min and 1.2 ml/min.
2. Column oven temperature (+ 2 °C) 27°C.

Solution Stability

Solution stability was performed for standard solution and sample solution up to 24 hours.

RESULT AND DISCUSSION

System Precision

	AREA RESPONSE		
	Dextromethorphan HBr	**Phenylephrine HCl**	**Chlorpheniramine Maleate**
Injection 1	2497518	1676314	716614
Injection 2	2488174	1665110	713348
Injection 3	2474277	1670892	713648
Injection 4	2478596	1669046	713492
Injection 5	2425895	1666157	713669
Injection 6	2444216	1682725	712915
Mean	2468112.67	1671707.33	713947.67
SD	27453.3	6703.6	1334.9
% RSD	**1.11**	**0.40**	**0.19**

The system is precise for assay of Dextromethorphan HBr, Phenylephrine HCl and Chlorpheniramine Maleate Syrup formulations.

Specificity

Blank and Placebo interference

Blank Interference	No peak was present at the retention time of each drug component in the blank chromatogram
Placebo Interference	No peak was present at the retention time of each drug component in the placebo chromatogram

Forced degradation study for Dextromethorphan HBr

Stress Condition	% Assay	%Degradation	Minimum Peak Purity Index	Peak Purity Pass/Fail
As such sample	103.7	-	552	Pass
Acid Degradation	103.1	0.6	237	Pass
Alkali Degradation	102.6	1.1	265	Pass
Heat Degradation	103.0	0.7	1046	Pass
Forced Heat and Humidity Degradation	102.6	1.1	225	Pass
Oxidative Degradation	101.9	1.8	171	Pass

Forced degradation study for Phenylephrine HCl

Stress Condition	% Assay	%Degradation	Minimum Peak Purity Index	Peak Purity Pass/Fail
As such sample	98.6	-	1677	Pass
Acid degradation	99.9	-1.3	556	Pass
Alkali degradation	42.6	56	4835	Pass
Heat degradation	98.1	0.5	2887	Pass
Heat and humidity degradation	98.2	0.4	580	Pass
Oxidative degradation	96.1	2.5	379	Pass

Forced degradation study for Chlorpheniramine Maleate

Stress Condition	% Assay	%Degradation	Minimum Peak Purity Index	Peak Purity Pass/Fail
As such sample	102.3	-	4788	Pass
Acid degradation	101.0	1.3	1955	Pass
Alkali degradation	100.4	1.9	1915	Pass
Heat degradation	100.7	1.6	7663	Pass
Heat and humidity degradation	100.3	2.0	1691	Pass
Oxidative degradation	95.8	6.5	972	Pass

The method is specific and stability indicating for assay of Dextromethorphan HBr, Phenylephrine HCl & Chlorpheniramine Maleate in Syrup formulation.

Method Precision

	% Assay		
	Dextromethorphan HBr	**Phenylephrine HCl**	**Chlorpheniramine Maleate**
Sample 1	101.4	100.2	97.5
Sample 2	103.2	100.3	97.8
Sample 3	102.2	101.0	97.3
Sample 4	104.2	101.1	98.7
Sample 5	101.3	98.4	96.0
Sample 6	101.5	98.7	96.1
Mean	102.3	100.0	97.2
SD	1.2	1.2	1.0
% RSD	**1.1**	**1.2**	**1.0**

The method is precise for assay of Dextromethorphan HBr, Phenylephrine HCl & Chlorpheniramine Maleate in Syrup formulation

Accuracy

Accuracy study for Dextromethorphan HBr

Recovery level	Amount added (µg)	Amount Recovered (µg)	% Individual Recovery	% Mean Recovery
Recovery at 80% prep-1	80.19	80.56	100.5	101.3
Recovery at 80% prep-2	80.19	81.46	101.6	
Recovery at 80% prep-3	80.19	81.64	101.8	
Recovery at 100% prep-1	100.24	101.04	100.8	101.2
Recovery at 100% prep-2	100.24	101.27	101.0	
Recovery at 100% prep-3	100.24	101.89	101.6	
Recovery at 120% prep-1	120.29	122.28	101.7	101.5
Recovery at 120% prep-2	120.29	122.28	101.7	
Recovery at 120% prep-3	120.29	121.61	101.1	

Accuracy study for Phenylephrine HCl

Recovery level	Amount added (µg)	Amount Recovered (µg)	% Individual Recovery	% Mean Recovery
Recovery at 80% prep-1	40.18	40.50	100.8	101.3
Recovery at 80% prep-2	40.18	40.77	101.5	
Recovery at 80% prep-3	40.18	40.88	101.7	
Recovery at 100% prep-1	50.22	49.80	99.2	100.9
Recovery at 100% prep-2	50.22	51.13	101.8	
Recovery at 100% prep-3	50.22	51.11	101.8	
Recovery at 120% prep-1	60.26	60.75	100.8	101.2
Recovery at 120% prep-2	60.26	61.06	101.3	
Recovery at 120% prep-3	60.26	61.24	101.6	

Accuracy study for Chlorpheniramine Maleate

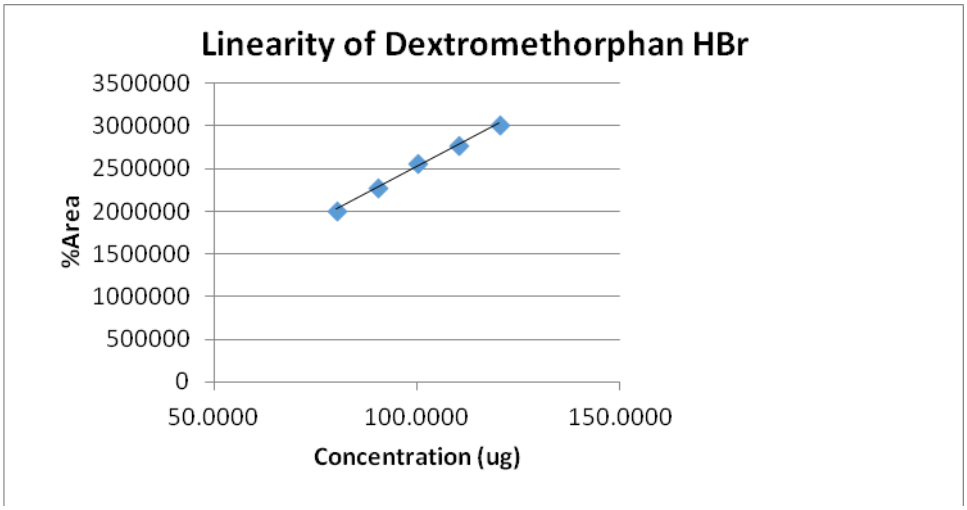
Recovery level	Amount added (µg)	Amount Recovered (µg)	% Individual Recovery	% Mean Recovery
Recovery at 80% prep-1	16.04	16.05	100.1	101.1
Recovery at 80% prep-2	16.04	16.33	101.8	
Recovery at 80% prep-3	16.04	16.28	101.5	
Recovery at 100% prep-1	20.05	20.18	100.6	101.3
Recovery at 100% prep-2	20.05	20.35	101.5	
Recovery at 100% prep-3	20.05	20.39	101.7	
Recovery at 120% prep-1	24.06	24.48	101.7	101.6
Recovery at 120% prep-2	24.06	24.46	101.7	
Recovery at 120% prep-3	24.06	24.42	101.5	

The method is Accurate in range of 80% to 120% of target concentration for the assay of Dextromethorphan HBr, Phenylephrine HCl & Chlorpheniramine Maleate in Syrup formulation.

Linearity

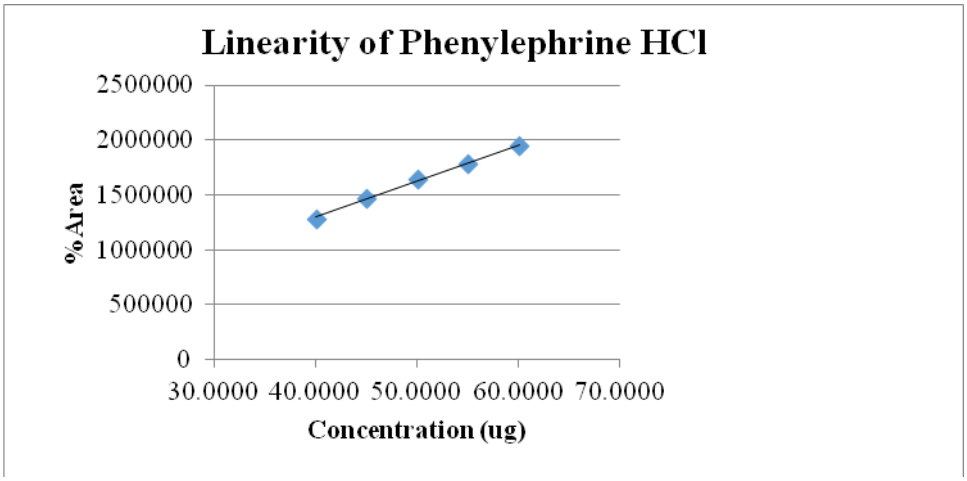
Linearity for Dextromethorphan HBr

Linearity Levels	Concentration Obtained (µg)	Peak Area
Linearity at 80%	80.23	2012706
Linearity at 90%	90.26	2275829
Linearity at 100%	100.29	2558853
Linearity at 110%	110.31	2778861
Linearity at 120%	120.34	3020220
Slope		25107.79
Intercept		11233.80
Correlation Coefficient		**0.999**



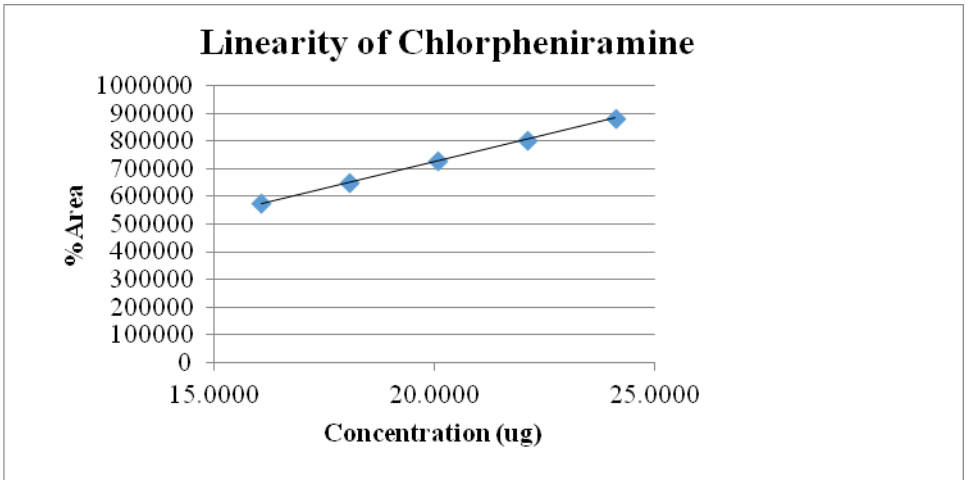
Linearity for Phenylephrine HCl

Linearity Levels	Concentration Obtained (µg)	Peak Area
Linearity at 80%	40.048	1284042
Linearity at 90%	45.054	1464831
Linearity at 100%	50.060	1647099
Linearity at 110%	55.066	1780334
Linearity at 120%	60.072	1948237
Slope		32838.45
Intercept		-18984.40
Correlation Coefficient		**0.999**



Linearity for Chlorpheniramine Maleate

Linearity Levels	Concentration Obtained (µg)	Peak Area
Linearity at 80%	16.076	575773
Linearity at 90%	18.085	651989
Linearity at 100%	20.094	728536
Linearity at 110%	22.104	805518
Linearity at 120%	24.113	882981
Slope		38216.24
Intercept		-38985.60
Correlation Coefficient		**1.000**



The method is linear in range of 80% to 120% of target concentration for the assay of Dextromethorphan HBr, Phenylephrine HCl & Chlorpheniramine Maleate in Syrup formulation.

Ruggedness (Intermediate precision)

	% Assay		
	Dextromethorphan HBr	**Phenylephrine HCl**	**Chlorpheniramine Maleate**
Sample 1	101.9	97.9	97.7
Sample 2	102.9	97.5	98.0
Sample 3	103.0	98.2	98.0
Sample 4	102.3	97.6	96.7
Sample 5	103.1	97.9	97.2
Sample 6	102.3	97.2	97.1
Mean	102.6	97.7	97.5
SD	0.5	0.4	0.5
% RSD	**0.5**	**0.4**	**0.5**
Overall % RSD (Total twelve preparations: six from method precision and six from intermediate precision)	**0.89**	**1.45**	**0.81**

The method is rugged for the assay of Dextromethorphan HBr, Phenylephrine HCl & Chlorpheniramine Maleate in Syrup formulation.

Robustness

Robustness for Dextromethorphan HBr

Parameters		**% RSD**	**Tailing Factor**	**Theoritical Plates**
Flow rate (mL/min)	As such condition	0.5%	1.64	31416
	Upper variation	0.1%	1.59	26640
	Lower variation	0.9%	1.71	36555
Column Oven (°C)	As such condition	0.5%	1.64	31416
	Upper variation	0.1%	1.63	30255

Robustness for Phenylephrine HCl

Parameters		% RSD	Tailing Factor	Theoritical Plates
Flow rate (mL/min)	As such condition	0.1%	1.36	3306
	Upper variation	0.1%	1.37	2814
	Lower variation	0.1%	1.34	3573
Column Oven (°C)	As such condition	0.1%	1.36	3306
	Upper variation	0.1%	1.32	3105

Robustness for Chlorpheniramine Maleate

Parameters		% RSD	Tailing Factor	Theoritical Plates
Flow rate (mL/min)	As such condition	0.3%	1.49	27107
	Upper variation	0.6%	1.38	20992
	Lower variation	0.6%	1.57	32684
Column Oven (°C)	As such condition	0.3%	1.49	27107
	Upper variation	0%	1.37	23560

The method is found to be robust in below conditions-
Column oven temperature: 25°C to 27°C
Flow rate: 0.8 to 1.2 mL

Solution Stability

Solution stability for Dextromethorphan HBr

Standard Preparation	Initial Area	Day 1 Area	Similarity Factor
	2515717	2526714.67	1.0
Sample	Initial (% Assay)	Day 1 (% Assay)	Difference
Sample 1	103.7	103.8	-0.1

Solution stability for Phenylephrine HCl

Standard Preparation	Initial Area	Day 1 Area	Similarity Factor
	1613119	1570219.33	1.02
Sample Preparation	Initial (% Assay)	Day 1 (% Assay)	Difference
Sample 1	98.6	99.5	-0.9

Solution stability for Chlorpheniramine Maleate

Standard Preparation	Initial Area	Day 1 Area	Similarity Factor
	737536	736248.67	1.01
Sample Preparation	Initial (% Assay)	Day 1 (% Assay)	Difference
Sample 1	102.3	102.3	0.0

Standard and sample solutions are stable up to 24 hours at bench top conditions.

CONCLUSION

A stability indicating method is developed and validated as per ICH guideline (Q2R1). From the above discussion, it can be concluded that the proposed method is specific, precise, accurate, linear, robust and rugged. There is no interference from execipients and degradant products. This method can be used for routine analysis of Dextromethorphan HBr, Phenylephrine HCl & Chlorpheniramine Maleate in Syrup dosage form.

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